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S/N 08/403,844

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	FODSTAD ET AL.	Examiner:	G. GABEL
Serial No.:	08/403,844	Group Art Unit:	1641
Filed:	APRIL 18, 1995	Docket No.:	7885.33USF1
CPA FILED:	JUNE 23, 1998		
Due Date:	NOVEMBER 25, 1999		
Title:	METHOD FOR DETECTION OF SPECIFIC TARGET CELLS IN SPECIALIZED OR MIXED CELL POPULATION AND SOLUTIONS CONTAINING MIXED CELL POPULATIONS		

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on November 24, 1999.

By: Brenda House
Name: Brenda House

RESPONSE

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This responds to the Office Action mailed 25 May 1999 in the above-identified application. In that Office Action, the Examiner withdrew claims 41-42, 73-74, 80-86, 90-91, 94-95, 97-100, and 102-104 from further consideration in this case. Claims 22-25, 28-29, 33-40, 43, 46-48, 51, 59-62, 64, 66-67, 69, 71-72, 75, and 78-79, 87-89, 92-93, 96, 101, and 105-107 are under consideration in this case.

This responds to the Office Action mailed 25 May 1999. Accordingly, a Petition under 37 C.F.R. §1.136(a) for an extension of time of three (3) months is enclosed herewith, along with a check in the amount of $435.00 to cover the required fees under 37 C.F.R. §1.17. If the Office determines that any additional fees are required for timely submission of the present Amendment, it is hereby authorized to charge such deficiency in fees to Deposit Account No. 13-2725.

In the Office Action mailed 25 May 1999, the Examiner made the election requirement final and withdrew claims 41, 42, 73, 74, 80–86, 90, 91, 94, 95, 97, 98 and 102 from further consideration under 37 C.F.R. 1.142(b) as being drawn to non–elected species. The Examiner withdrew the rejection of claims 29, 61 and 78–79 under 35 U.S.C. §112, second paragraph. Further, the Examiner rejected claims 22–25, 28, 29, 33 and 105 under §103(a); rejected claims 22, 34–40, 43, 48, 67, 69, 71, 72, 75, 87–89, 92, 93, 96 and 101 under §103(a); and rejected claims 22, 46–48, 51, 59–62, 64, 66, 67, 69, 71, 78, 79, 106 and 107 under §103(a). Applicants respectfully disagree with each of the rejections under 35 U.S.C. §103(a) and respectfully request reconsideration.

The Examiner rejected claims 22–25, 28, 29, 33 and 105 under 35 U.S.C. §103(a) as being unpatentable over *Widder et al.* (EP 016,552) in view of *Connelly et al.* (USP 5,422,277). Applicants respectfully disagree and request reconsideration.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: 1) there must be some suggestion or motivation to modify the reference or to combine the references; 2) there must be a reasonable expectation of success; and 3) the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142. Applicants submit that *prima facie* obviousness has not been established, as there is no reasonable expectation of success, and the cited references do not teach or suggest all of the claim limitations. Thus, even if one of skill in the art were to combine or modify the references as indicated by the Examiner, he or she would not achieve the present invention. The presently claimed invention provides a method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from solid tissue. The presently claimed method involves coating paragmagnetic particles with monoclonal antibodies specific for a membrane structure present on target cells alone, forming target-cell-bead rosettes, and quantitating the rosettes. Even if one of skill in the art were to modify the references as indicated by the Examiner, he or she would not have any reasonable expectation of success in achieving the present invention. Moreover, neither of the references, alone or in combination, teaches or suggests the present invention.

The Examiner conceded that the method of *Widder* differs from the instant invention in failing to teach incubation of the antibody coated microspheres in mild detergent for 5 to 10 minutes to 2 hours at 4° C. Further, the Examiner stated that *Widder et al.* fail to teach the use of

an antibody to immobilize antibodies on the surface of magnetic particles. However, in addition to these deficiencies, *Widder et al.* fail to teach detection of an individual target cell as claimed in the present application. *Widder et al.* disclose a method for coarse separation of blood cells, not detection of individual target cells. Applicants refer to previous arguments made in communications filed 23 June 1998 (at pages 13-15 of the preliminary amendment) and 25 February 1999 (at pages 2-3).

Widder discloses microspheres having protein A associated with the outer surfaces thereof. Due to the use of protein A, the microspheres of *Widder* will adhere to non-target cells and target cells alike and cause unacceptable reduction in specificity. In addition to binding the Fc portion of IgG cells, protein A will also bind to B cells and plasma cells present in a sample. As a result, *Widder* cannot achieve the present invention, since this method cannot detect a specific target cell in a cell suspension. Further, there is no teaching or suggestion in *Widder* of forming target cell-bead rosettes as claimed in the present application which can then be quantified.

In the Office Action, the Examiner stated that the *Widder* method was combined with the method of *Forrest* and monoclonal antibodies of *Terasaki*. However, as discussed in more detail below, these references do not cure the deficiencies of the *Widder* reference.

Moreover, even if a person with knowledge in the art should consider using monoclonal antibodies on the particles, on which *Widder* used protein A, he or she would not have a reasonable expectation of success. To the contrary, he or she would still expect that the method would have the same disadvantages typical for *Widder's* method. The Examiner stated that it would have been obvious to one of ordinary skill in the art to use antibodies to immobilize other antibodies on the surface of the magnetic particles in the method of *Widder* because such method of immobilizing antibodies on the surface of a solid support, such as magnetic particles, is conventional and well known in the art. However, there is no reasonable expectation of success. The present inventors have surprisingly succeeded in avoiding the unspecific binding characterizing *Widder* by using the presently claimed method. The inventors further use the target cell-bead rosettes to visually detect the cells using a microscope. This type of detection has not been accomplished previously, since it has not been possible to obtain particle binding exclusively to target cells. In fact, the present inventors encountered criticism from expert

scientists in the field that the present invention would suffer the same disadvantages as encountered in *Widder et al.*

Widder et al. separates cells when only two types of cells are present by using radioactive chromium, while the idea of the present invention is to detect individual target cells, the detection being done by visualizing the target cell rosettes under a microscope. The present inventors have surprisingly succeeded in avoiding the unspecific binding characterizing the other cited methods. Further, the inventors can use the particle-target-cell rosette to visually detect the cells under the microscope. Many antibody-coated beads attach to the target cell, thereby creating a rosette that is easily detected using, for example, a microscope. This was not possible previously, because it had not been possible to obtain particle binding exclusively to a specific target-cell. Thus, it would not have been obvious to one or ordinary skill in the art to modify *Widder* as indicated by the Examiner.

Connelly teaches a cell fixative composition for fixing the internal components of a cell without disrupting the cell surface components. *Connelly* fails to cure the deficiencies of *Widder*. *Connelly* uses fixatives without destroying the cells structurally, but these cells were killed. The present invention examines live cells, and these cells can be viewed in a microscope without using fixatives at all. The present method suggests optionally using fixatives if in special cases further visualization or characterization steps after detecting the cells are warranted. *Connelly* does not address detection methods for target cells as presently claimed.

The Examiner stated that it would have been obvious to one of ordinary skill in the art to use detergents to treat cells as used by *Connelly* following certain specific temperature and time parameters because the use of detergents to treat cells is well known and conventional in the art for removing extraneous matter from the cells that will interfere with assays. However, the use of detergents does not necessarily increase the specificity of the detection method. Thus, one of skill in the art would have no reasonable expectation of achieving the present invention by using detergents as indicated by the Examiner. Persons with knowledge in the art would not consider using detergents since it would not make *Widder's* method more specific. In fact, the inventors of the presently claimed invention attempted to use detergents without obtaining sufficient specificity. However, use of a combination of detergents and low concentration and low temperatures surprisingly gave the specificity of the presently claimed method.

In contrast, the present invention teaches a method for detecting a specific target cell in a cell suspension. The method involves coating paramagnetic particles or beads with an antibody or antibody fragment directed against a membrane structure specifically expressed on the target cell and not on non-target cells in the cell mixture. As a result, any type of non-specific binding, such as that taught in *Widder*, is unacceptable in the present invention. Moreover, the claimed method involves incubating the mixture of coated paramagnetic particles or beads with the cell suspension until target cell-bead rosettes are formed.

Therefore, even if one of skill in the art were to combine the teachings of *Widder* and *Connelly*, he or she would not achieve the present invention. The references do not teach or suggest, alone or in combination, a method for detecting a specific target cell in a cell suspension, wherein beads are coated with antibody directed against specific membrane structures expressed on the target cells only, and not on non-target cells in the cell suspension, and wherein target cell-bead rosettes are formed and quantitated as described and claimed in the present application. Also, one of skill in the art would have no reasonable expectation of success in achieving the present invention by making the modifications indicated by the Examiner.

The Examiner rejected claims 22, 34-40, 43, 48, 67, 69, 71, 72, 75, 87-89, 92, 96 and 101 under 35 U.S.C. §103(a) as being unpatentable over *Widder et al.* in view of *Kemmner et al.* (Journal of Immunological Methods) and *Holmes et al.* (WO 91/09938) and further in view of *Terasaki et al.* (USP 4,752,569). *Widder* is discussed above and Applicants refer to the above discussion as it applies to this rejection. Again, *Kemmner*, *Holmes* and *Terasaki* fail to cure the deficiencies of *Widder*. Applicants respectfully submit that *prima facie* obviousness has not been shown for this rejection. The combination of the cited references does not teach or suggest all of the limitations of the present claims. Also, one of skill in the art would not have a reasonable expectation of success.

Although *Holmes* was not discussed in the present outstanding Office Action, the Examiner stated in Paper No. 9 that *Holmes* et al. teach a method of separating haemopoietic progenitor cells from a mixed population of haemopoietic cells which contain malignant cells using magnetic microbeads coated with sheep-anti mouse antibody which binds to the Fc portions of IgG mouse antibodies or protein A which reacts universally with the Fc portion of virtually all IgG antibodies. To the extent the Examiner maintains the arguments of Paper No. 9

with respect to *Holmes* in the present rejection, Applicants will address this reference. Notwithstanding, Applicants respectfully disagree with each of these rejections and request reconsideration.

Kemmner and *Holmes* each teach non-specific methods, while *Terasaki* teaches hybridoma cell lines. None of the references, alone or in combination, teach or suggest a method for detecting a specific target cell using the presently claimed method.

Kemmner teaches the use of beads for enriching a cell population prepared from a solid tumor containing mainly tumor cells, and the reference uses the bead/cell complex to assess the effects of the enrichment. However, despite the high number of malignant cells in relation to normal cells in the reference, the results of the method provided heterogenic cells, including tumor cells. In fact, only 96% of the bead-rosetting cells with the specific antibody proved to be tumor cells and 5% of the cells attached to the beads coated only with an irrelevant antibody bound to tumor cells (see page 199, column 1 of the reference). Moreover, of the 34% cells that bound control beads coated with an antibody recognizing the human leukocyte common antigen (Dako-LC), as much as 35% turned out to be tumor cells (see page 199, column 1 of the reference). This is a good example of the unspecificity of *Kemmner's* method since leukocyte common antigen is not expressed on the tumor cells. These data demonstrate a highly unspecific method which cannot be used to specifically and reliably detect a specific target cell in a mixed cell population.

Holmes also provides a nonspecific method for separating haemopoietic progenitor cells from a mixed population of haemopoietic cells. *Holmes* provides a combination of positive and negative selection wherein both procedures can be performed in opposite succession and the beads are detached and removed from the cells. The method is performed with haemopoietic cells, and there is no required specificity. The positive selection antibody is taught as being reactive with a broadly expressed HPC antigen, e.g., an antibody reactive with the CD34 antigen. *Holmes* goes on to state that, "[M]ore broadly active antibodies are also of value since the negative selection step will remove unwanted cells included within the wider antigen groupings and leave only the desired HPC" (see page 5, lines 17–23). Other examples of positive selection antibodies include a pan class II specific IgM antibody, and an IgG which also recognizes a monomorphic DR antigen on stem cells. Similarly, the negative selection antibody is

nonspecific. Thus, *Holmes* provides a nonspecific method that uses antibodies found on a variety of cell types. There is no teaching or suggestion of a method of detecting a specific target cell as claimed in the present application.

Terasaki teaches using hybridoma cell lines for producing antibodies for detection of an epitope found in neoplastic cells, as well as a significant number of normal tissues (see column 2), and does not refer to binding antibodies to cells. Further, *Terasaki* teaches detection of free antigen in the blood. In this case, all cells are removed from the sample. Therefore, *Terasaki* adds nothing to the other references in this case.

There is no reasonable expectation of success when modifying or combining the references discussed above. *At best*, one of skill in the art might expect to achieve a method with the level of non-specificity as taught in *Widder*. In such a method, non-target-cells would be included in the rosettes and the method would not be useful as a diagnostic measure. In *Holmes*, the number of particles bound to non-target-cells would be the same as the number of particle bound to target-cells, because sub-groups of normal cells will express the target antigen on their surfaces. There is no reasonable expectation that a method of detecting a specific target cell can be achieved by combining/modifying the references.

In contrast to these references, the present invention provides a method for detecting a specific target cell in a population of millions of cells. The present method involves coating paramagnetic particles with a monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell mixture, and forming target cell-bead rosettes that are quantitated. None of the references, alone or in combination, teaches or suggests the method as claimed. As discussed above, *Widder* simply teaches a coarse separation of blood cells which is nonspecific and provides low sensitivity. Moreover, *Kemmner* and *Holmes* also suffer from non-specificity. Finally, the addition of *Terasaki* with the above references does nothing to cure the deficiencies. Therefore, even if one of skill in the art were to combine these references, he or she would not achieve the present invention; i.e., detection of a specific target cell in a cell suspension, whereby paramagnetic particles or beads coated are with a monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell mixture, wherein target cell-bead rosettes are formed and quantitated.

The Examiner rejected claims 22, 46-48, 51, 59-62, 64, 66, 67, 69, 71, 78, 79, 106 and 107 under 35 U.S.C. §103(a) as being unpatentable over *Widder et al.* in view of *Forrest et al.* (USP 4,659,678). *Widder* is discussed above and Applicants refer to the above discussion as it applies to this rejection. *Forrest et al.* also lacks elements of the present invention, and its combination with *Widder* does not cure the deficiencies of either reference. The references fail to teach or suggest all of the claim limitations; therefore, *prima facie* obviousness has not been shown.

The Examiner stated that it would have been obvious to one of ordinary skill in the art to use a binding system such as avidin-biotin as taught by *Forrest et al.* in the method of *Widder et al.* because *Forrest et al.* teach that avidin-biotin provides a very rapid and high binding affinity which offers the advantage of a more accurate and rapid assay. Further, the Examiner stated that it would have been obvious to one of ordinary skill in the art to use a binding system used by *Forrest et al.* in the method of *Widder et al.*, as modified by *Forrest et al.* in a test kit arrangement because test kits are conventional and well known in the art.

However, as the Examiner herself conceded, *Forrest et al.* teach a sandwich assay involving two or more antibody reagents; the use of Protein A attached to the solid support and further attached to an antibody; and the use of antibody reagents that constitute a specific binding protein such as avidin and biotin. There is no teaching or suggestion of the presently claimed method, i.e., a method of detecting a specific target cell in a cell suspension using paramagnetic particles or beads coated with a monoclonal antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell suspension, forming target cell-bead rosettes, and quantitating the target cell-bead rosettes.

Moreover, *Forrest* does not cure the deficiencies of *Widder*. Even if one of skill in the art were to combine the teachings of these references, he or she would not achieve the presently claimed method. There is no teaching or suggestion of the highly specific detection method presently claimed.

In view of the remarks presented herein, Applicants respectfully submit that the claims are in condition for allowance. Notification to that effect is earnestly solicited. If prosecution of this case could be facilitated by a telephonic interview, the Examiner is encouraged to call the undersigned.

Respectfully submitted,

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